Comp Clin Pathol
DOI 10.1007/s00580-012-1555-x

ORIGINAL ARTICLE

Group B streptococcus colonization of pregnant women: comparative molecular and microbiological diagnosis

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Received: 29 March 2012 / Accepted: 27 June 2012
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Abstract This study aimed to compare the sensitivity of different culture methods from three different anatomic sites and to evaluate the sensitivity of polymerase chain reaction (PCR) assay targeting the 16 S ribosomal RNA gene in detection of group B streptococcus (GBS) colonization in pregnant women. From 100 pregnant women at 35–37 weeks of gestation, three cotton swabs were used to obtain vaginal, rectal, and rectovaginal (RV) specimens and plated onto Columbia agar with colistin and nalidixic (CNA), group B streptococcus differential agar (GBSDA), and chromID Strepto B agar (CA), with and without Lim broth enrichment. PCR assay was done on the RV swabs. The overall GBS colonization rate was 29 % by culture and 31 % by PCR. GBS positivity for RV sampling (100 %) was significantly higher than detection on the basis of vaginal sampling (50 %), but not significantly higher than for rectal sampling (82 %). Direct plating of the rectovaginal swab on CNA, GBSDA, and CA resulted in detection of 74, 58, and 100 % of the carriers, respectively, whereas subculturing of Lim broth yielded 65, 59, and 83 % positivity, respectively. Using GBS culture as the "gold standard," the sensitivity of PCR was 100 %, and specificity was 97 %. We found that the inoculation of RV secretions directly onto CA is the most rapid, easy, and sensitive method than that of Lim broth enrichment. Also, we found that group B streptococci can be detected rapidly and reliably by a PCR assay of rectovaginal secretions from pregnant women.

Keywords PCR · GBS · Rectovaginal · Culture

The study was done in Cairo University, Egypt.

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Introduction

Streptococcus agalactiae, group B streptococcus (GBS), is a significant cause of perinatal and neonatal infections worldwide. Rectovaginal colonization occurs in 10 to 30 % of pregnant women (Anthony et al. 1978; Boyer et al. 1983a; Dillon et al. 1982) and is responsible for 1.8 neonatal infections per 1,000 live births per year (CDC 1996a).

It is possible to get infected with GBS during labor or in utero by transmission from maternal vaginal or anorectal colonized mucosa. There is another risk factor for GBS neonatal sepsis, which is prematurity, and mortality because GBS is higher in preterm compared to the number of newborns (Benitz 2002).

Because results at 35–37 weeks correlate more closely with GBS colonization at term delivery, the Centers for Disease Control and Prevention (CDC) has recommended that all pregnant women be screened for carriage of GBS between 35 and 37 weeks of gestation (CDC 2002), so that GBS positive women can receive antibacterial treatment (chemoprophylaxis) prior to delivery, to reduce mother-to-child transmission. Nowadays, prenatal screening by culture, in broth culture or selective medium, is considered the gold standard method for detection of anogenital GBS colonization (Dillon et al. 1982).

To maximize GBS carriage detection rates, both the anatomic site of sampling and the culture methods used are important. Rectovaginal swabs have been reported to provide high bacterial yields, as the gastrointestinal tract is a natural reservoir for GBS and a potential source of vaginal colonization (CDC 2002; Dunne and Holland-Staley 1998; El Aila et al. 2009a; Philipson et al. 1995).

Published online: 24 July 2012

GBS colonization status may be transient, chronic, or intermittent, and the duration of colonization varies; therefore, the diagnosis of GBS in the vagina at the certain points in pregnancy cannot assure the existence of this organism at the time of delivery (Boyer et al. 1983b). However, since culture takes 24 to 72 h, this method would be of limited value as guidance for antimicrobial prophylaxis when specimens for culture are collected at delivery. Many techniques have been tested in order to validate a fast and efficient method of GBS screening to replace the culture; molecular biology based assays, such as polymerase chain reaction (PCR) tests, have become the focus of investigation of detection of GBS colonization in pregnant women (Bergseng et al. 2007). Therefore, this study aimed to evaluate the sensitivity and specificity of different culture for detecting GBS carriage in pregnant women and, additionally, to evaluate the PCR performance as GBS colonization screening in pregnant women.

Methods

Study design

The study was approved by the Research Ethics Committee of Cairo University Hospital, Egypt, and all the women gave oral informed consent. Between April 2011 and June 2011, 100 vaginal, 100 rectal, and 100 rectovaginal (RV) cotton swab samples were collected from 100 pregnant women at 35–37 weeks of gestation.

Collection of the specimens

Rectovaginal, vaginal, and rectal samples were collected using cotton swabs that were submerged into 1 ml of Amies transport medium (Oxoid, England). Rectovaginal sampling was carried out by rotating swab against the vaginal wall at the midportion of the vault. Subsequently, the swab was carefully withdrawn to prevent contamination with microflora from the vulva and introitus, and the swab was inserted 1.5 to 2 cm beyond the anal sphincter and gently rotated to touch the anal crypts. Next, vaginal sampling was carried out by inserting the swab, following the same procedure described above for swabbing the vaginal wall.

The third swab was used for rectal sampling as described above for the anal procedure of the rectovaginal sampling. All samples were collected by doctors and transported to the main laboratory of Kaser Alini within 4 h.

Culture of specimens

Direct plating was carried out only for the rectovaginal swab by inoculating 50 μl from the swab transport medium onto Columbia agar with 5 % sheep blood and with 10 mg/ml colistin and 15 mg/ml nalidixic acid (CNA, Becton Dickinson, Erembodegem, Belgium), 50 μl onto group B streptococcus differential agar (GBSDA) (Becton Dickinson), and 50 μl onto chromID Strepto B agar (CA) (BioMérieux, Marcy l'Etoile, France). The CNA plates were incubated at 37 °C in 5 % CO_2 for 24–48 h; the GBSDA plates were incubated at 37 °C in an anaerobic chamber (Oxoid anaerobic jars) for 24–48 h, and the CA plates were incubated at 37 °C for 18–24 h in aerobic conditions in the dark.

Volumes of 200 μl from the swab transport medium of the rectovaginal, vaginal, and rectal swabs were inoculated into separate tubes with 5 ml of Todd-Hewitt broth with 1 % yeast extract, 15 μg/ml nalidixic acid, and 10 μg colistin/ml (Lim broth, Becton Dickinson), which were incubated aerobically at 37 °C and subcultured onto CNA, GBSDA, and CA after overnight incubation. GBSDA was examined for yellow orange pigment colonies indicative of the presence of GBS, whereas CA was examined for pale pink to red, round, and pearly colonies. B- and nonhemolytic colonies were picked from CNA for further identification. The isolates were confirmed as *S. agalactiae* using the API 20 strept for identification of the isolate to the species level (BioMérieux, Marcy l'Etoile, France) (Madani et al. 1998).

Extraction of DNA from bacteria on RV swabs

Swabs were soaked in 1 ml of phosphate-buffered saline (pH 7.2) and agitated vigorously to dislodge bacteria and epithelial cells. Extraction of DNA was performed using the QIAamp DNA mini kit (50) (QIAGEN GmbH, Hilden, Germany; Cat. No. 51304).

PCR primers

The selected PCR oligonucleotide primers were designed from highly divergent and species-specific regions of the DNA coding for 16 S rRNA (Riffon et al. 2001).

Specificity	Sequences (5′–3′)	Size of product amplified (bp)
S. agalactiae (f)	CGCTGAGGTTTGTGTTTACA	405
S. agalactiae (r)	CACTCCTACCAACGTTCTTC	

Preparation of PCR master mix (total volume 25 μl)

Each PCR consisted of 2.5 μl of 10× buffer (without $MgCl_2$), 2 mM of $MgCl_2$, 0.4 mM dNTPs, 0.2 μM of each primer, 1 U of Taq DNA polymerase, 5.0 μl of DNA template/sample, and distilled H_2O to a total volume of 25 μl. The PCR amplification was performed as described in Riffon et al. (2001).

Table 1 Number of GBS culture-positive samples detected by different culture media in RV, V, and R specimens obtained from 29 GBS-positive women

Specimen	CNA	GBSDA	CA	SCNA	SGBSDA	SCA	Estimated number of women colonized
RV swab	21	17	29	19	17	24	29
V swab	NT	NT	NT	20	21	18	21
R swab	NT	NT	NT	13	16	16	16

CNA Columbia agar with colistin and nalidixic acid, *GBSDA* group B streptococcus differential agar, *CA* chromID Strepto B agar, *NT* not tested, *RV* rectovaginal, *V* vaginal, *R* rectal

PCR amplification

The amplification reactions were done in the thermocycler (Master cycler 5330, Hamburg). A pre-PCR step at 94 °C for 2 min was applied. A total of 35 PCR cycles were run under the following conditions: denaturation at 94 °C for 45 s, annealing at 60 °C for 1 min, and extension at 72 °C for 2 min. After the final cycle, the preparation was kept at 72 °C for 10 min to complete the reaction The PCR products were stored in the thermocycler at 4 °C until they were collected.

Detection of PCR products

To confirm amplicon production, the mixture (10-µl PCR product and 2 µl of loading buffer) was analyzed by electrophoresis in 1.5 % agarose gel stained with ethidium bromide. Electrophoresis was carried out in 1× TAE (40 mM Tris-acetate, 1 mM EDTA; pH 8.0) at 100 V for 45 min. The DNA molecular weight marker (Boehringer Manheim, Germany), 100-bp DNA ladder, was run concurrently. Gels were visualized under UV illumination, and the results were photographed.

Results

A total of 300 swabs were taken from 100 pregnant women at 35–37 weeks of gestation, and the age was ranging from 22 to 32. Twenty-nine were identified as carriers of GBS on the basis of the results of culture of RV specimens, as compared with 21 on the basis of culture of vaginal specimens and 16 on the basis of culture of rectal specimens (Table 1). The main complaint was vaginal discharge in 68 %; history of neonatal complication was in 5 % of them, and no one of them gave a history of previous antibiotic intake.

The GBS detection rate on the basis of rectovaginal samples (29 GBS positive women) was significantly higher than the detection rate on the basis of vaginal samples (21 positive) (P=0.01) and significantly higher than that on the basis of rectal samples (16 positive) (P=0.12).

When direct plating of the RV swabs on CNA, GBSDA, and CA of the total number of carriers was carried out, a result of (21/29) 74 %, (17/29) 58 %, and (29/29) 100 %, respectively, was detected on all samples and media. The subculture onto on CNA, GBSDA, and CA resulted in positivities: (19/29) 65 %, (17/29) 59 %, and (24/29) 83 %, respectively. For all sampling methods and for RV swab with and without subculture CA and CNA, more positive women were detected in comparison with GBSDA.

Altogether, the culture method turned out positive in 29 (29 %) samples, while the PCR technique was positive in 31 (31 %) (Table 2). PCR results of representative positive and negative samples, along with molecular weight standards as shown in Fig 1. All culture-positive samples were also positive with the PCR technique, therefore resulting in 100 % PCR sensitivity (95 % confidence interval (CI) 91.62–100). Of the 71 culture-negative samples for GBS, two were positive with PCR, and 69 were negative with both methods, which indicate a specificity of 97.2 % (95 % CI 81.79–90.71) of the molecular method. The negative predictive value was 100 %, and positive predictive value was 93.5 %. An excellent agreement beyond chance (Kappa) between the techniques was 0.952.

In general, group B streptococci were detected slightly more often by the PCR assays than by culture (Table 2). It requires 3–4 hours to obtain the results of the conventional PCR assay, while 24–72 h should be passed for culture.

Statistical analysis

Sensitivity, specificity, PPV, and NPV were calculated for the PCR technique using culture as gold standard (Table 3).

Table 2 Comparison between PCR and culture of rectovaginal specimens

PCR	Culture (RV)		Total
	Positive	Negative	
Positive	29	2	31
Negative	0	69	69
Total	29	71	100

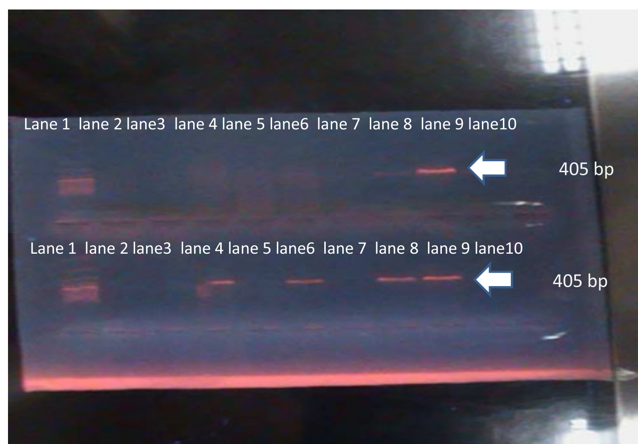

Fig. 1 The agarose gel electrophoresis of the PCR using the 16S rDNA primer. *Panel* (*1*) in upper half indicates *lanes: 1* DNA marker, *2–8* negative cases, *9* positive cases with a band at 405 bp, while *10* negative case. *Panel* (*2*) in lower half indicates *lanes* 1 DNA marker; *4*, *6*, *8*, and *9* positive cases; *2*, *3*, *5*, *7*, and *10* negative cases

The concordance between assays was determined using the Kappa coefficient. The statistical analysis was performed in SPSS version 13.0.

Discussion

The current recommendation to prevent group B streptococcal disease in neonates is to screen pregnant women by culture of RV secretions at 35 to 37 weeks gestation and to treat those with positive cultures or to treat women with risk factors for disease transmission empirically (CDC 1996b).

In comparison of the three samples, we found that RV swabbing was the best sampling method to detect GBS colonization of pregnant women because the highest percentage was 29 % GBS-positive women, and those were detected by RV sampling .Our results correspond with previous reports that GBS colonization of rectovaginal samples is 18 % to 24 % higher than that of vaginal samples (Madani et al. 1998; Quinlan et al. 2000) and more appropriate than vaginal sampling only (Dunne and Holland-Staley 1998; Allen et al. 1993); Diaz and Neives 2008).

Although our results are in correspondence with the CDC recommendations to carry out RV sampling, it should be noticed that Nomura et al. (2006) found no significant difference in detection rates between vaginal and rectal samples. Gupta and Briski (2004) reported a similar detection rate of 23.8 % of GBS when using RV and vaginal sampling.

Different types of selective media to improve GBS isolation have been described. Islam (1977) showed that adding horse serum and starch to agar-based media increased the orange/red pigment formation that was already present to some degree on Columbia agar and was typical for GBS, being absent for all other serotypes. De La Rosa et al. (1983) improved this medium when added horse serum at 90 to 95 °C instead of at 55 °C. This led to agar to be opaque and increased the natural pigmentation of the colonies, specific for *S. agalactiae*.

Usage of the correct starch and proteose peptone n°3 in addition of the folate inhibitor trimethoprim (15 μg/ml) would strongly affect pigmentation, especially in the presence of anaerobic atmosphere. This medium was designated Granada medium (GM). The same authors later improved GM, which they designated as new Granada medium (NGM) (de la Rosa et al. 1992).

Increasing orange to salmon pigmentation of the colonies is done by use another folate synthesis which is methotrexoate (6 μg/ml), and also by adding 0.2 μg/ml crystal violet, 5 μg/ml colistin sulfate, and 10 μg/ml metronidazole made the agar selective media. It should be noted that horse serum was added again at 55 °C.

In our study, we used modification of NGM which is the commercially available group B streptococcus differential agar that improve stability and selectivity. Its usefulness has been evaluated in several studies (El Aila et al. 2009b; Bou et al. 2005; Adler et al. 2008). Bou et al. (2005) found that the intensity of colony pigmentation on GBSDA is stronger than on GM, and that the commensal microflora is more suppressed.

Some studies found comparable sensitivity of direct plating on "Granada Medium" compared with Lim broth enrichment (Boyer et al. 1983b; de la Rosa et al. 1992; Rosa-Fraile et al. 1999), whereas other studies found direct

Table 3 Sensitivities, specificities, and positive and negative predictive values for the six different culture methods, based on all 300 samples and calculated for a number of 29 positive subjects on a total number of 100 subjects included

Culture medium	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
CNA	72	94	72	89.3
GBSDA	58.6	97	58	85
CA	100	98.5	96	98
Lim broth + CNA	65	98.2	92.8	94.6
Lim broth + GBSDA	58	98.7	94.7	95.4
Lim broth + CA	82	99.5	96.6	97

plating on chromogenic and/or selective media significantly less sensitive (Gupta and Briski 2004; Regnath and Ignatius 2009). Blanckaert et al. (2003) suggested using a combination of Granada and Columbia blood agar and an adequate sample (RV swab in transport medium) for optimal GBS screening.

Our data suggest that CA is faster, sensitive, and easier to use than the CDC-recommended method for the detection of GBS, in agreement with several other recent studies. Also, Tazi et al. (2008) found that, compared to CA and GBSDA, Lim broth enrichment enabled the detection of only two additional samples leading to 34 (17 %) GBS-positive cultures. Also, in the study of Bou et al. (2005), only one swab was only positive, following subculture in Lim broth, and was missed on direct GBSDA (Tazi et al. 2008).

By direct plating on CA provided high sensitivity for GBS detection among pregnant women and provided superior recovery of GBS when compared with GBSDA and CNA. This is in agreement with another study (Gupta and Briski 2004), which showed that selective media, producing pigmented colonies, are more sensitive in GBS detection than enriched media like blood agar or selective media like CNA. Direct plating on CA and GBSDA offers the advantage of reducing workload and providing an identification of GBS 24 h sooner than the Lim broth enrichment method.

In our study, all GBS isolated from CA and GBSDA were identified within 1–2 days of specimen receipt, whereas all Lim broth enrichment cultures required a minimum of 2–3 days for the identification of specimens positive for GBS. In addition, CA offers an additional advantage with respect to GBSDA because culture on CA can be carried out aerobically, not requiring special equipment and extra costs and workload associated with anaerobic culture needed for GBSDA.

Comparing of GBS detection with culture versus PCR of RV specimens, the sensitivity and negative predictive value of the PCR assay was 100 %; the specificity was 97.2 %, and the positive predictive value was 93.5 % (Table 2). In our study, two women revealed positive GBS by PCR and not by culture; hence, the false positive rate was 2 % (2/100). These deviations can be resulted from the existence of nonviable bacteria in the vaginal swabs or unequal sample collection between the swabs obtained from pregnant women. RV specimens contain nonviable bacteria which cannot grow on media, but bacterial DNA would be present for PCR amplification (Islam 1977). Despite the fact that PCR is a sensitive and specific technique, the process of PCR assay increases the cost, and the technology of PCR is not usually available in all laboratories in our country. This means that the clear benefit of using CA culture media is the cost effectiveness in less amount of time. A limitation of this study may be the cost and the limited sample size of 100 subjects, although it should be noted that each subject was studied intensively.

Conclusion

In conclusion, we found that GBS can be detected rapidly and reliably by a PCR assay of rectovaginal secretions from pregnant women, and because of the fluctuation of GBS colonization during pregnancy, PCR could be used as a screen test at time of labor for high-risk patients. The inoculation of RV secretions directly onto CA is the most rapid, easy, and sensitive method than that of Lim broth enrichment. Also, direct inoculation offers several advantages, such as decreased workload because no subculture is needed and decreased time to detection, i.e., at least 24 h faster than the standard method. The specificity problems associated with the use of CA can be resolved by confirmation of CA-positive isolates with the API test.

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